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NUTRITION REVIEWS

VOL. 16 NOVEMBER 1958 No. 11

NUTRITION OF MAMMALIAN CELLS IN TISSUE CULTURE

Ideally, considerations of the nutritional requirements of any species embraces both qualitative and quantitative aspects. The availability of newer techniques and materials has made possible studies of cells in tissue culture which are concerned with both of these. Extensive investigations in utilizing newer techniques in this connection were those of Eagle (*Science* **122,** *501 (1955)*; *Arch. Biochem. Biophys.* **61,** *356 (1956)*) using cells of strain HeLa, derived from a human carcinoma, and strain L mouse fibroblasts. (Of the recent extensive literature which has accumulated in the field of tissue culture nutrition, work published up to the end of 1956 is reviewed in *Bact. Rev.* **22,** *20 (1958)* by Morgan.)

Thirteen amino acids, eight vitamins (thiamine, riboflavin, pantothenate, nicotinamide, pyridoxine, inositol, choline, and folacin), glutamine, glucose, and six inorganic ions (Na^+, K^+, Mg^{++}, Ca^{++}, Cl^-, and phosphate) were found essential in addition to a small amount of dialyzed serum. The omission of any one of these nutrients arrested multiplication and caused cellular degeneration which ultimately resulted in the loss of all cells. Reversal of the deficiency was possible provided it had not advanced too far. Satisfactory concentrations for these materials were found to be approximately 0.02 to 0.2 mM for the amino acids and 0.1 to 1 micromolar for most of the vitamins. In many instances considerably higher concentrations decreased the rate of cellular multiplication. *Added* bicarbonate was not required and the requirement for a number of nutrients such as biotin and vitamin B_{12} could not be demonstrated. As Eagle has pointed out, the dialyzed serum probably contributed other nutrients. Trace elements would also be introduced in the salts employed. According to Puck and co-workers (*Science* **126,** *961 (1957)*) the size of the cell inoculum is also an important factor in such nutritional studies.

Glucose has been more extensively used in tissue culture media than any other carbohydrate. Galactose, mannose, and maltose were effectively utilized by strain L fibroblasts and HeLa cells (Eagle, *loc. cit.*). Mannose, fructose, galactose, maltose, starch, and glycogen substituted for glucose in the nutrition of HeLa and conjunctival cells, and with certain sera, sucrose was also metabolized (R. S. Chang and R. P. Geyer, *Proc. Soc. Exp. Biol. Med.* **96,** *336 (1957)*). Rueckert and Mueller (*Proc. Am. Assoc. Can. Research* **2,** *340 (1958)*) observed that cells of the HeLa strain utilized glucose, mannose, fructose, galactose, maltose, turanose, and trehalose. At the present time, available evidence indicates that the utilization of di- and polysaccharides by cells in tissue culture is dependent upon the carbohydrases in the serum used. Rigorous exclusion of glucose contamination is, of course, essential in all studies of carbohydrate requirements.

Eagle, Oyama, Levy, and Freeman (*Science* **123,** *845 (1956)*) reported that inositol could replace serum dialysate in the nutrition of a variety of human cells *in vitro*. However, those of the HeLa strain gave inconsistent results. Subsequently, Eagle, *et al.* (*J. Biol. Chem.* **226,** *191 (1957)*) and Geyer and Chang (*Proc. Soc. Exp. Biol. Med.* **95,** *315 (1957)*) showed that a consistent requirement for inositol could be demonstrated for this strain as well as for the human conjunctival strain of cells. A variant of strain L fibroblasts also has been shown to require inositol by Swim and Parker (*Spec. Publ. N. Y. Acad. Sci.* **5,** *351 (1957)*).

Bicarbonate was found by Geyer and

Chang (*Arch. Biochem. Biophys.* **73,** *500 (1958)*) to be required for the net multiplication of cells of the HeLa and conjunctiva strains. To demonstrate the requirement, it was necessary to incubate the cells in the presence of an absorbing agent to remove CO_2 from both endogenous and exogenous sources. Cells depleted of CO_2 resumed net multiplication when the absorbing agent was removed. In agreement with the earlier report of Eagle (*Arch. Biochem. Biophys.* **61,** *356 (1956)*) no advantage could be assigned to *added* HCO_3^-. Harris (*J. Exp. Zool.* **125,** *85 (1954)*) showed that the lack of CO_2 arrested fibroblast outgrowth from chick embryo hearts. The mode of action of the CO_2 has not been elucidated.

The amino acid requirements and metabolism of the cells of several strains have continued to receive a great deal of attention. In this respect, the presence of dialyzed serum in the media employed has been of particular inconvenience and has necessitated studies to determine the fate of the serum proteins themselves. Conflicting results in this regard have been obtained, but recent studies with labeled proteins indicate that little splitting occurs and that the proteins themselves may be taken up by the cells and incorporated as such (J. F. Morgan, *Bact. Rev.* **22,** *20 (1958)*; H. N. Kent and G. O. Gey, *Proc. Soc. Exp. Biol. Med.* **94,** *205 (1957)*). The latter workers have shown that a significant decrease occurs in the concentration of serum alpha and beta globulins during incubation with cells. A necessary component of serum protein appears to be in the α-globulin fraction (G. Sato, H. W. Fisher, and T. T. Puck; *Science* **126,** *961 (1957)*; I. Lieberman and P. Ove, *Biochem. Biophys. Acta* **25,** *449 (1957)*). Fetuin, the α_1-globulin fraction of fetal serum protein, has also been found to be highly active (H. W. Fisher, T. T. Puck, and G. Sato; *Proc. Nat. Acad. Sci.* **44,** *4 (1958)*). Puck and co-workers believe it is possible that the mode of action of the α-globulin is to inhibit proteolytic action.

Malignant and normal cells require valine, lysine, methionine, leucine, isoleucine, histidine, glutamine, cystine, arginine, phenylalanine, threonine, and tyrosine (H. Eagle, V. I. Oyama, and M. Levy; *Arch. Biochem. Biophys.* **67,** *432 (1957)*). The requirement for tryptophan varied among the strains, and persistent survival in its absence was often obtained. Both tissues *in vitro* and cells *in vivo* have approximately the same amino acid pool (A. Piez and H. Eagle, *J. Biol. Chem.* **231,** *533 (1958)*). Levintow and co-workers (*J. Biol. Chem.* **227,** *929 (1957)*) have demonstrated that glutamine is incorporated into cell (HeLa) protein without change and that it acts independently from glutamic acid. Studies by Thomas, *et al.* (*Science* **127,** *591 (1958)*) indicate that arginine becomes a limiting factor during incubation and that supplementing the medium periodically with this amino acid eliminates the necessity to change media every few days as is customarily done.

In contrast to cells of human derivation, several strains of mouse cells multiply for extended periods of time in the absence of serum protein. Thus, Waymouth (*J. Nat. Can. Inst.* **17,** *315 (1956)*) has grown strain L fibroblasts in chemically defined media plus a peptone preparation. Rabson and associates (*Nature* **181,** *1343 (1958)*) have adapted P388 mouse cells to grow on a medium comprised of chemically defined materials plus autoclaved fat-free milk. Earle and co-workers (*Canad. Research* **16,** *77 (1956)*; *J. Nat. Can. Inst.* **19,** *885 (1957)*) obtained prolonged survival and multiplication of a strain L clone culture in media which are chemically defined. Under these conditions, 12 amino acids were found essential (Sanford, *et al.*; *J. Nat. Can. Inst.* **20,** *775 (1958)*). Healy, Fischer, and Parker (*Proc. Soc. Exp. Biol. Med.* **89,** *71 (1955)*) have successfully grown strain L mouse fibroblasts for more than four months using a synthetic-type medium. It is of interest that in the absence of serum approximately 60 compounds are employed whereas, in the

presence of dialyzed serum, less than half this number are used.

A number of variants have been intentionally obtained which possess altered nutritional requirements. Puck and Fisher (*J. Exp. Med.* **104,** *427 (1956)*) have shown this with respect to the HeLa strain, and Swim and Parker (*Spec. Publ. N. Y. Acad. Sci.*, **5,** *351 (1957)*) obtained a number of nutritional variants of fibroblasts. Likewise, Chang (*Proc. Soc. Exp. Biol. Med.* **96,** *818 (1957)*) has found variants of human cells which can utilize xylose, lactate, and ribose in place of glucose.

Unintentional selection of cells by nutritional selection or other means offers adequate reason for differences to arise between stocks of a given strain, thus resulting in conflicting experimental findings. The widespread use of antibiotics in tissue culture media may also be a cause for concern. An additional factor which may play a role in affecting results is that of the actual sterility of the cells employed. Robinson, Wickelhausen, and Roizman (*Science* **124,** *1147 (1956)*) found pleuropneumonia-like organisms (PPLO) in a number of cell strains even though the usual signs of bacterial contamination were absent. The possible effects of such contamination on nutritional and biochemical investigations are disquieting. It has not always been clear from published reports whether or not the cells used had been checked for the presence of PPLO.

As a fuller understanding of the nutrition of mammalian cells continues to emerge, many of the seeming limitations will appear in proper perspective. The successful propagation *in vitro* of many different cells of both normal and malignant origin, and the use of cells growing from fresh tissue explants, allow a wide selection of experimental material. Recent advances in cultivation techniques and improved refined media make studies of the nutritional requirements of mammalian cells in tissue culture an important part of the broad field of nutrition.

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GENETIC ASPECTS OF METABOLIC DISEASE IN CHILDHOOD: PART I

During the last few years specific knowledge concerning the enzymatic defects in a number of metabolic diseases appearing in early life has made it possible to identify the basic mechanisms in a number of congenital metabolic diseases. This information has permitted some estimation of the genetic aspects of these diseases by appropriate studies in the families of such patients. Consequently, genetic metabolic disease is assuming a role of increasing importance in the field of pediatrics since a large number of these diseases involve the metabolism of well known nutrients and may, in some cases, be benefited by specific nutritional therapy. A brief general review should be of interest to nutrition workers in many fields.

A symposium on this subject under the chairmanship of C. U. Lowe (*Pediatrics* **21,** *1018 (1958)*) was recently presented before the American Academy of Pediatrics. The genetic aspects of these diseases were discussed by B. Childs, who points out that the genetic etiology of any characteristic is established by finding this characteristic in more than one member of a family tree.

Although specific measurements directly related to the action of a single gene are known for the blood-group antigens and the hemoglobin variants (*e.g.* Sickle Cell Anemia), appropriate tests are showing similar mechanisms to be present in a number of other metabolic diseases such as familial, non-hemolytic jaundice. In the

latter condition, patients become jaundiced immediately after birth and remain so as long as they live. There is no hemolysis or liver damage. The ability of these patients to excrete bilirubin as a glucuronic acid conjugate was found to be defective, and their parents and some of their siblings also showed some impairment of this conjugating mechanism. In other genetic enzymatic defects there may be no overt expression except under certain specific circumstances.

Individuals having a reduced activity of glucose-6-phosphate dehydrogenase in their red cells are unusually susceptible to hemolysis when exposed to such substances as Permaquine, naphthalene metabolites, vitamin K, and a factor in fava beans. *In vitro* testing methods have been devised to demonstrate this defect in individuals who have never shown hemolytic anemia. Studies using these methods have demonstrated that this is a genetic disorder associated with a sex-linked, incompletely dominant gene.

In other instances, several wholly dissimilar biochemical functions may result in a common disease picture. Such is the case in goitrous cretinism, in which three separate blocks in the biosynthesis of thyroid hormone may occur and can be differentiated only by specific metabolic studies. Similar situations may be responsible for some of the confusion in our understanding of other disease conditions suspected of being of genetic origin. A more detailed discussion by Childs and J. B. Sidbury, Jr. has been published (*Pediatrics* **20,** *177 (1957)*).

Because of its susceptibility to modification by diet therapy phenylpyruvic oligophrenia, or phenylketonuria, is of great current interest. A. Miester (*Pediatrics* **21,** *1021 (1958)*) has summarized the current knowledge concerning this disease. Phenylketonuria is characterized by mental deficiency and the urinary excretion of phenylpyruvic acid. The basic metabolic defect in this disorder is a failure in the conversion of phenylalanine to tyrosine. In this respect, the human mutants are analagous to those induced in *Neurospora crasa* and *E. coli*, except that the human defect is relatively complex. The abnormal excretory products in this disease are phenylpyruvic and phenyllactic acids, as well as large quantities of phenylalanine itself. Phenylacetylglutamine and phenylacetic acid may also be present and there is some evidence that two tryptophan derivatives, indolacetic and indolelactic acids, are also present in some cases, suggesting a similar but milder defect in the oxidization of indole compounds derived from tryptophan.

The most specific metabolic change in this condition is the accumulation of phenylalanine in the blood. There is evidence that the oxidative deamination of this amino acid proceeds via transamination reactions. However, there is no reason to think that this mechanism depletes the body of significant amounts of glutamic acid. The best evidence for implicating the relationship between high blood phenylalanine levels and brain damage is the improvement which occurs, even in older patients, when a low-phenylalanine diet is given. The blood levels of this amino acid return to normal levels. A definite requirement for phenylalanine exists and enough has to be added to the deficient diet to maintain normal serum concentrations and to promote growth.

Metabolic studies in patients with this relatively uncommon disease have yielded information concerning the nutritional requirements for phenylalanine, as well as elucidating several steps in the intermediary metabolism of the aromatic amino acids.

The adrenogenital syndrome, or pseudohermaphroditism in the female (a condition simulating hermaphroditism in which the external sexual characteristics are of male aspect, but ovaries are present) and macrogenitosomia praecox (precocious development with large genitals) in the male, has been found to be a congenital defect in the synthesis of hydrocortisone. Current thinking on this problem is reviewed by A. M. Bongiovanni (*Pediatrics* **21,** *1031 (1958)*). A

series of studies has demonstrated that the basic defect in this disease is in the introduction of an oxygen atom at the 11, 17 and 21 positions in the steroid molecule. The failure of hydrocortisone production, and consequent low blood levels, stimulate ACTH production and the adrenal gland continues to produce large quantities of the intermediary metabolites of hydrocortisone, some of which have strong androgenic action. That this process begins before birth is demonstrated by the virilism and altered genitalia of affected females. Treatment with hydrocortisone or related products permits the restitution of normal adrenal and pituitary relationships.

Actually, three forms of this disease have been described and the specific intermediary metabolites have been characterized fairly well. Present evidence would suggest that three different genetic loci and different enzymatic mechanisms are involved. Although most patients have the uncomplicated form, several with a hypertensive form due to the accumulation of metabolites with compound S activity have been described. An even greater number of patients have been reported in whom sodium and chloride loss is a major manifestation. In these latter patients the evidence would suggest that the defect was more severe, although a specific salt-losing hormone had been predicated at one time.

Another type of congenital defect is also associated with the excessive loss of sodium and chloride from the body. The recent description of the high sweat electrolyte concentration in fibrocystic disease of the pancreas (*Nutrition Reviews* **14,** *41 (1956)*) has led to further studies which indicate that this is a very constant finding. Studies on the parents of patients would suggest that this aspect of the disease might become useful in tracing the genetic patterns which have long been suspected on the basis of clinical data alone.

Summary: Congenital disease accounts for an increasingly large proportion of the population of most pediatric wards. It has been estimated that almost one third of the patients in a children's hospital at any one time have been admitted for the diagnosis or treatment of some form of congenital disease. This trend is due to both the reducing incidence of infectious disease, and the availability of specific treatment for congenital disease. The increasing recognition of the genetic basis of metabolic disease is also responsible for this changing population under pediatric care.

[Part II of this review will appear in the January 1959 *Nutrition Reviews*.]

BIOLOGICAL LIFE OF VITAMIN B_{12}

With the development of radioisotopic tracer methods it has been possible to measure the turnover rates of many biologic compounds, and some have proven to be far more rapid than had been surmised. Clinical evidence has long suggested that vitamin B_{12} might have a much longer life than that of some other vitamins.

L. L. Schloesser, P. Deshpande and R. F. Schilling have reported on the "Biological turnover rate of cyanocobalamin (vitamin B_{12}) in human liver" (*A.M.A. Arch. Int. Med.* **101,** *306 (1958)*). They point out that most relapses in pernicious anemia occur more than six months after treatment is stopped and 25 per cent of relapses more than a year later. Similarly, megaloblastic anemia after total gastrectomy requires a few years to develop. One measurement of serum cyanocobalamin after total gastrectomy, quoted by the authors, showed a 50 per cent decrease in nine months. Earlier studies on the liver have usually concerned radioactivity after injection of Co^{60}-labeled

cyanocobalamin. Schloesser and his colleagues point out that possible biologically inactive Co^{60}-containing degradation products are not distinguished by such radiation measurements. They offer evidence, by biologic assay and chromatography, that they have indeed measured cyanocobalamin.

The authors studied six men with pernicious anemia and one healthy man. The dose and details of the protocol are not stated, except that "all subjects studied had received cyanocobalamin Co^{60} orally several months prior to the beginning of these observations" (plus intrinsic factor) and that "the patients were receiving 30 to 100 micrograms of cyanocobalamin every three or four weeks as maintenance therapy." Radioactivity over the liver was compared to the reference sample at the start of the experiment and the ratio was considered to be 100 per cent. Later determinations were plotted semi-logarithmically against time. The liver of one patient who received cyanocobalamin Co^{60} 11 months before death from chronic lymphocytic leukemia was fractionated for the vitamin. Various fractions were tested for radioactivity and the cyanocobalamin content was assayed biologically. The final liver extract was compared with known cyanocobalamin by paper chromatography.

The biologic half life of radioactivity in the liver was about 400 days, both in the normal subject and in the pernicious anemia patients receiving maintenance doses. One of the latter, after 217 days, was given 1 mg. of cyanocobalamin intramuscularly daily for seven days; this was associated with a sharp fall in hepatic radioactivity, presumably a "flushing" effect. The radioactivity and cyanocobalamin content of the various fractions of the liver specimens were approximately parallel (ratio 2.6 to 4.4 counts per minutes, per microgram of cyanocobalamin). The red spot and radioactivity of the purified extract moved chromatographically as in the known sample of the vitamin.

The disappearance curves of hepatic radioactivity are nearly straight on the semi-logarithmic plots. The authors have fairly well established that they are measuring cyanocobalamin and not some other Co^{60}-containing derivative. It is true, that the latter might be present in some degree, but it seems very unlikely that it would fractionate chemically and chromatographically so like the vitamin as to be indistinguishable. Likewise there is no reason to believe the natural vitamin behaves differently from the labeled.

It is interesting to speculate on the molecular function of cyanocobalamin, needed by the body only in amounts of the order of 1 microgram per day, largely kept in the liver, and only half gone at the end of a year. It is hard to imagine a substance so sparsely distributed and so well conserved by the cells it serves, yet of such potency and importance to the bodily economy.

In summary, the biologic half life of cyanocobalamin in the liver is about 400 days.

PROTEIN REQUIREMENTS OF CHILDREN

Those who have attempted to assay the protein requirements of children have found themselves faced with a much more complex problem than is generally realized. The difficulties range from lack of suitable experimental subjects to individual variation, and from choice of criteria to influences of childhood diseases and growth rates. Investigations must take place over the course of many years during which time it is continuously difficult to establish whether the child is on a subminimal, minimal or optimal protein diet and even more difficult to regulate the protein in the diet once criteria have been chosen.

Three groups of workers in this vital, if

complex, field of endeavor have recently contributed the results of over half a decade of investigation to a Symposium on Protein Nutrition sponsored by the New York Academy of Sciences (G. Stearns, K. J. Newman, J. B. McKinley and P. C. Jeans, *Ann. N. Y. Acad. Sci.*, **69,** *857 (1958)*; H. C. Stuart, B. S. Burke, R. B. Reed, and I. Valadian *Ibid.* **69,** *869 (1958)*; J. A. Johnston *Ibid.* **69,** *881 (1958)*).

The data reported by Stearns *et al.* (*loc. cit.*) include 458 studies of 51 children 1 to 4 years of age and 481 studies of 67 children 4 to 11 years of age. Most of the children were from orphanages and were males. It was found (by nitrogen analysis and estimations from tables) that the childrens' mean caloric intake matched the recommended allowances very closely despite the fact that each child's caloric intake was determined largely by his own appetite. The mean protein intake as a percentage of total caloric intake varied from 16 per cent for 1- and 2-year-olds to 13 per cent for 10-year-old boys, all well above allowances recommended by the National Research Council.

The requirement of a child for a given nutrient is accepted by the authors as that intake which will permit the rate of growth and development which is "normal" for the child's age. This rate is defined by the authors as the mean growth of children not restricted in the intake of any essential nutrient and protected against adverse influences of environment. The protein requirement at any age will depend upon the resultant of the rate of growth and the nitrogen content of each tissue at that age. Although the rate of growth of tissues other than muscle is more difficult to measure, it is known that considerable skeletal musculature can be sacrificed during periods of protein deprivation to prevent loss of protein from more vital tissues. Thus it can be assumed that the protein requirements of other body tissues have been met if the skeletal musculature is well maintained. The authors stress the rate of growth of muscle as the dominating criterion for determining the protein requirement of children. Since the amount of creatinine excreted in 24 hours is an excellent measure of the amount of skeletal musculature, the authors have chosen this method as the best for measurement of growth.

It was found that the excretion of creatinine during somewhat more than the first half of the second year was maintained at the same relatively low rate. During the latter part of the second year, the excretion rose with almost startling abruptness. This increase in relative quantity of muscle occurred during the period of most marked slowing of growth in body weight and height. After the second year, the amount of creatinine excretion increased at a steadier rate.

Nitrogen was well absorbed at every age studied. The mean fecal nitrogen remained constant at about 11 per cent of the intake. Of the protein absorbed no significant difference in retention for any given intake could be noted among children aged 1 to 4 years. The retention of nitrogen by children aged 4 to 11 years was somewhat lower and less constant than that of infants given comparable intakes. Curiously, small children (1 to 4 years) retained very little of a low nitrogen intake, but the retention rose sharply between 400 and 500 mg. nitrogen intake per kg., then reached a plateau, only to show a second rise when the intake was increased above 650 mg. of nitrogen. Children 4 through 10 years of age showed no significant differences in mean quantity of retention for a given nitrogen intake.

Except for one group of 24 studies of 5-year-olds, whose mean nitrogen intake was 309 mg. per kg., the intake of the 4- to 7-year-old children lay predominantly between 475 and 600 mg. of nitrogen per kg. of body weight. The nitrogen intake of children 7 to 11 years of age was distributed more evenly, almost 80 per cent being between 325

and 525 mg. per kg., with the total range of 225 to 661 mg. per kg. With increasing protein intake, nitrogen retention of the 4 to 11 year-old children tended to increase stepwise, as did that of the younger group. Furthermore, all showed some evidence of resisting loss of nitrogen at low intakes. The retention of the older group leveled off at 46 mg. of nitrogen per kg. of body weight between intakes of 300 and 400 mg. of nitrogen per kg. of body weight.

Some orphanage diets provided protein in the amount of about 2 to 2.5 g. per kg. per day. These children had poor musculature. When those under seven were given a diet containing 3 g. per kg. protein (approximately 15 to 16 per cent of the total calories taken) they maintained an excellent musculature. The authors claim that an intake above 3.25 g. per kg. per day is uneconomical for this age group. They conclude that an intake of 3 g. of protein per kg. for the 4- to 7-year-olds, decreasing gradually as the child grows older but keeping well above 2.5 g. per kg. per day, will permit maintenance of growth of muscle and allow sufficient protein for recovery from the illnesses common to children.

In a study carried out by Stuart *et al.* (*loc. cit.*) during the past 26 years, 134 individual children have been followed periodically in a multi-disciplinary manner from birth to 18 years of age. Primary emphasis was placed on the recognition of individual differences, in patterns of growth and development on the one hand and in dietary intakes, illness experiences, and environmental circumstances on the other. Two case studies were presented in detail. One subject was able to consume levels of calories and of protein far above recommended allowances while remaining relatively inactive and maintaining low weight for height, excellent health and a high level of progress in growth. In contrast, a second boy was always small and continously consumed small intakes of both calories and protein. Both intakes were very low in comparison with the major group in this study and the protein component was particularly low with respect to National Research Council allowances. Progress in skeletal development became slowed during this period; his health was only fair and he displayed a generally lowered resistance to infections. The authors suggest that it may be more appropriate to consider the adequacy of an older child's intake in terms of his previous customary levels of intake while enjoying good health, or in terms of his progress in growth, than in relation to standard allowances.

J. A. Johnston (*loc. cit.*) has considered the problem of "optimal" intake of protein in adolescents. Criteria which have previously been used in experimental animals include optimal growth, highest fertility, lowest mortality of offspring and length of life. These objectives are difficult to attain in any longitudinal study of the human being and may not be appropriate. Therefore, the author has studied the relation of protein intake to resistance to infection, specifically tuberculosis and rheumatic fever. In the case of active tuberculosis the typical metabolic picture included abnormally high nitrogen retentions on adequate intakes with flat or declining weight curves. Recovery from depletion was characterized by a high nitrogen storage with a plateau in weight curve following which nitrogen storage fell to the expected plane and was paralleled by normal weight gain. In a series of studies on children with rheumatic fever, good correlation was established between the nutritional state (protein reserves) and the ultimate outcome of the disease. A study was made on normal adolescents in which the per cent of protein in the diet was varied and the criteria used for adequacy of intake was (1) expected gain in weight and height, (2) satisfaction of appetite, (3) nitrogen retention and (4) a normal basal metabolism. In girls, cessation of menses is a side effect. Inability to cope with infection is an additional consequence. The author concludes

from this experience the "optimal" intake of protein for the adolescent should constitute 15 per cent of an adequate caloric intake. The author suggests that the recommendations of the National Research Council for the adolescent be revised upward.

A critical evaluation of the data presented emphasizes again the great difficulty and complexity of assessing the optimal protein intake. For example, equal nitrogen retentions of approximately 110 mg per kg. per day were obtained in children between one and three years of age. There are enormous differences in the rate of gains of infants between these ages, and calculation will demonstrate that if 110 mg./kg is gained by a three-year-old, his total protein retention will exceed his weight gain during the year. Similarly a retention of 80 mg. N/kg/day in a four to seven-year-old child is equivalent to more protein than his expected weight gain, which is about five pounds per year. Many balance studies available for other nutrients as well as protein yield values that are almost certainly erroneous. It is probable that systematic cumulative positive errors are responsible. Newer methods of determining changes in body composition may aid in the clarification of this problem. Certainly care must be used in assessing nutritional requirements from such data.

PROTEIN REQUIREMENTS IN CHRONIC RENAL DISEASE

One gram of protein per kilogram of body weight is the recommended intake of the Food and Nutrition Board proposed for the adult individual not in the pregnant or lactating state (*Recommended Dietary Allowances*, revised 1958, National Academy of Sciences, National Research Council, publ. #589). Actual measurements of nitrogen balance on minimal protein intakes indicate that balance is achieved with intakes of 30 to 40 per cent of this figure when the source of protein provides the necessary mixture of amino acids and adequate calories are provided. In certain clinical conditions it is important not to give this excess of dietary protein but only the amount necessary to maintain nitrogen balance or minimal nitrogen excretion. In chronic renal disease where the ability of the body to excrete the products of protein metabolism is limited, protein intakes in excess of actual requirements would seem undesirable.

The actual protein needs of patients with chronic renal insufficiency have been examined experimentally by R. F. Herndon, S. Freeman and A. S. Cleveland (*J. Lab. Clin. Med.* **52,** *235 (1958)*) to provide the basis of rational dietary treatment of azotemia. Careful nitrogen balance studies were carried out on five azotemic patients with protein intakes of 1.0 g., 0.5 g. and 0.25 g. of protein and 30 calories per kilogram of body weight. Adequate equilibration periods were used after changing the dietary protein intake before urine and fecal collections were made. This feature of the experiment would seem particularly important in these patients in whom delayed urinary excretion would slow equilibration. It was observed that all five patients were essentially in nitrogen balance on intakes of 1.0 g. per kilogram; persistent negative nitrogen balance occurred with intakes of 0.5 g. of protein per day when the losses of protein in the urine were included. Normal individuals achieve balance on this intake within about two weeks.

In actually estimating the minimum protein intake required to maintain nitrogen balance, use was made of the fact (*see* H. H. Mitchell, *Protein and Amino Acid Requirements of Mammals*, A. A. Albanese editor New York, *1950 Academic Press, Inc. p. 8*), that a linear relationship exists between the nitrogen balance and the net absorption of nitrogen when an individual is in more than

10 per cent negative nitrogen balance. This important correlation permits an extrapolation of the protein absorbed necessary to achieve balance. It was found that, excluding nitrogen losses from proteinuria, balance would be obtained with between 3.50 and 4.34 mg. of absorbed nitrogen per basal calorie. It is remarkable that the results were little altered by substitution of whole egg or egg albumin for mixed dietary protein. Also no striking lowering of minimal protein requirement was observed when the dietary protein was supplemented with the essential amino acids for human nutrition (Rose). The effects of adding glycine, L-leucine, L-isoleucine and L-methionine were also equivocal.

On the basis of these observations the authors indicate that the protein requirement of a mixed diet to maintain balance in chronic azotemia, exclusive of proteinuria was between 4.05 to 5.70 mg. nitrogen per basal calorie as compared to 3.8 mg. nitrogen per basal calorie in the normal subject studied. Expressed in more familiar terms, this amounts to between 0.54 and 0.70 g. per kilogram in uremia.

Thus it is concluded that in chronic renal disease the requirement for dietary protein is increased. The explanation for this abnormality was not established. It is noteworthy that the degree of disturbance in nitrogen metabolism failed to correlate with the usual indices of renal function. The authors point out that red cell survival is shortened in azotemia and suggest that the turnover of other body cells may also be accelerated accounting for the increased protein requirement.

In summary, metabolic balance studies have been carried out which apparently demonstrate an increased protein requirement for nitrogen balance in chronic renal failure. Therapeutic regimens utilizing the minimal protein intake of normal individuals may be expected therefore to lead to gradual protein depletion in the azotemic subject.

PROTEINS IN ANEMIA

In most parts of the tropical and subtropical world anemia continues to be an important cause of ill health despite intensive and usually successful antimalaria campaigns, and less successful onslaughts against hookworms (H. Foy, A. Kondi and A. Hargreaves., *Trans. Roy. Soc. Trop. Med. Hyg.* **46,** *327 (1952)*; H. Foy and A. Kondi, *J. Trop. Med. Hyg.* **60,** *105 (1957)*).

Although the anemias found in coastal areas and river valleys of India and Ceylon are predominantly of the iron-deficiency variety and respond to oral ferrous sulfate, it has been suspected that these, as well as the megaloblastic types which occur in the higher and cooler areas, may be predisposed or at least complicated by such protein deficiency states as marasmus and kwashiorkor.

While carrying out treatment and studies of Africans in the King George VI Hospital in Nairobi, Kenya, Foy and Kondi (*Trans. Roy. Soc. Trop. Med. Hyg.* **52,** *46 (1958)*) had opportunity to investigate this aspect of the anemia problem. Eighty five subjects were examined and treated, all having hemoglobin values below 8.5 g./100 ml. with a mean of 5.2 g./100 ml. (normal 14.0 g./100 ml.). There were 33 cases of megaloblastic and 23 of iron-deficiency anemia. The remaining 28 anemias were a miscellaneous collection which included splenic hemolytic, bleeding ulcers, gynecological and infectious.

It was found by the investigators that 58 per cent of the anemias among the Nairobi patients were megaloblastic as compared to 15 per cent previously found to be the case in Indian patients. The difference is thought by them to be due to the fact that the Africans were drawn from areas of 3000 to 6000 feet in altitude where iron-deficiency anemias are less prevalent because of cooler climates with

less sweating (*Nutrition Reviews* **15,** *294 (1957)*). In Africa, as contrasted to India, megaloblastic anemia was found by the authors to be nearly as frequent in males as in females.

The megaloblastic anemias of Africans were found to be of three main types: (1) folacin-responding, (2) penicillin or oral B_{12}-responding and (3) pernicious anemia.

Surprisingly, in such gross states of malnutrition as marasmus and kwashiorkor anemia was found to be rare, was never the presenting symptom, and was of no specific type. No response of megaloblastic anemia was noted when protein alone was administered to patients. The initial serum protein patterns and the changes that took place in them during treatment did not seem to be related in any way to the severity of the anemia or to the type of treatment, although variations in the α_1-, α_2- and β-globulin fractions during the course of illness were often marked. It is therefore the opinion of the authors that states of protein nutrition play little role in the etiology and cure of megaloblastic anemias.

There was little correlation between liver and spleen enlargement, diet and anemia. Liver biopsies further showed that erythrophagocytosis, anoxia, disparity in size and vacuolization of liver cells, thickening of the portal tracts or siderosis, were not correlated with anemia, proteins or responses to treatment. Nor was the serum B_{12} level associated with the presence or degree of cytological damage to the liver. Enlargement of the liver was not correlated with an abundance or lack of dietary protein or the proportion of it from animal sources.

The African B_{12}-deficient anemias responded fully in most cases to treatment with antibiotics such as penicillin. This may imply that the synthesis of essential substances such as B_{12}, folacin, pyridoxin, riboflavin, vitamin K, and others, by intestinal flora is an important factor in the prevention of this type of anemia. The flora themselves are determined by the type of food eaten and it is the speculation of the authors that the high bulky carbohydrate diet common in underdeveloped countries may produce intestinal flora inimical to the synthesis of hematopoietic substances or, perhaps, one that competes with the host for them. The megaloblastic anemia associated with infection from *Diphyllobothrium latum* is an example of the competition for B_{12} that can occur between host and parasite. The fact that, in Africa, megaloblastic anemia is nearly as frequent in males as in females further indicates that reproductive processes in the females are not primary factors in its etiology in Africa and suggest that the dietary and domestic habits of the African male render him more susceptible than his Indian counterpart to this type of anemia.

The serum proteins were also followed in 19 cases of iron-deficiency anemia. It was evident, as in the cases of megaloblastic anemia, that the blood cell values were not obviously related to changes in the protein picture. Siderophyllin (Transferrin) was estimated in a number of cases, during and after treatment, but, again, no consistent pattern was found which could be related either to the anemia or its treatment.

STUDIES ON DIETARY LIPIDS

The current wide interest in lipids and their role in nutrition has stimulated investigations that are contributing significantly to our knowledge. Two recent publications from the University of Western Ontario, London, Ontario, Canada, are of particular interest (K. K. Carroll, *J. Nutrition* **64,** *399 (1958)*; Carroll and J. F. Richards, *Ibid.* **64,** *411 (1958)*).

The studies reported by these workers are concerned with measuring the coefficient of digestibility of dietary fats (free

fatty acids, methyl esters of fatty acids and triglycerides).

Male rats, 80 to 90 g. in body weight, served as experimental animals. Digestibility data were based on two seven- to eight-day fecal collection periods on rats caged in groups of three. Purified diets were used to study the digestibility of free fatty acids. The basal diet had the following percentage composition: casein, 22; glucose, 63; fatty acid, 10; and salt mixture, 5. To these were added cellu flour (5 g. per 100 g.) and vitamins in amounts to satisfy requirements.

Fecal fat obtained on the experimental diets was corrected for metabolic fecal fat by feeding a basal diet composed of the following: casein, 19; glucose, 73; salt mixture, 4; and the cellu flour and vitamin supplement.

Fatty acid digestibilities of 100 per cent were obtained for the even numbered fatty acids containing four to ten carbon atoms (butyric through capric). With fatty acids of longer carbon chain length the digestibilities decreased progressively with stearic acid (C_{18}) and behenic acid (C_{22}), yielding digestion coefficients of 12 and 7 per cent, respectively.

Mono-unsaturated fatty acids were more digestible than their corresponding saturated acids with oleic (C_{18}) averaging 84 per cent and erucic (C_{22}) 53 per cent in digestibility.

Methyl esters of the fatty acids when tested in the same basal diet, were somewhat more digestible than the corresponding free acids.

The digestion coefficients are essentially in agreement with those reported for fatty acids by Deuel (*The Lipides*, Vol. II, Biochemistry, page 195, Interscience Publ., Inc., New York (1955)).

Carroll points out that the melting point of a fat cannot reliably serve as a prediction of its digestibility, as has been suggested by earlier studies (A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition* **37,** *237 (1949)*). Carroll points out that nervonic acid (mono-unsaturated C_{24} acid) has a melting point of 39° C. and therefore would probably be in liquid form in the digestive tract, whereas stearic acid melts at 69° C. and would be in solid form in the digestive tract. Both of these acids yielded about the same digestion coefficients.

The reliability of digestion information with regard to lipids is always complicated by the formation of insoluble soaps in the process of digestion. Carroll thus devoted some studies to methods for the extraction of fecal fat. A procedure proposed by V. Augar, H. S. Rollman and H. J. Deuel, Jr. (*J. Nutrition* **33,** *177 (1947)*) consists of desiccating feces in vacuo, subjecting them to overnight extraction with ether to remove neutral fat and free fatty acids, and extracting the residue with acidified (HCl) ether to remove the fatty acid present in soaps. This procedure was found to be unsatisfactory since ether extraction appeared to remove considerable quantities of a black gum or oil which was subsequently not soluble in petroleum ether.

A procedure proposed by C. Y. Hopkins, T. K. Murray, and J. A. Campbell (*Canad. J. Biochem. Physiol.* **33,** *1047 (1955)*) was adopted as a more reliable procedure. It consists of petroleum ether extraction of dry feces for neutral fat and free fatty acids, followed by treatment with five volumes of acetic acid in 100 volumes of petroleum ether for the removal of fatty acids present in soaps. Two to three extractions with the acetic acid-petroleum ether mixture were necessary, however, to insure complete removal of fatty acids in the soap fraction.

Further studies by Carroll and Richards (*loc. cit.*) were designed to compare the digestibilities of fatty acids and their corresponding triglycerides. Triolein and trilinolein had digestion coefficients of 99 and 97 per cent, respectively, contrasted with values of 73 and 84 per cent when the same free fatty acids were fed. The im-

provement in digestibility when these acids were fed as the triglycerides appeared to be due mainly to a reduction in the neutral lipids excreted in the feces.

Palmitic and stearic acids, when fed as free fatty acids, yielded most of the fecal lipid as soaps. When the same acids were fed as triglycerides, the fecal lipids were predominantly neutral fats. The authors conclude that these fatty acid triglycerides, which were somewhat less well digested than the corresponding free fatty acids, are poorly digested because they appear to be less readily hydrolyzed during passage through the digestive tract. On the other hand, the improvement in digestibility of trierucin over erucic acid could be ascribed to a reduction in the amount of fatty acid appearing as soap in the feces.

That minerals (calcium and phosphorus) in the diet may play an important role in lipid utilization from the diet was emphasized. The salt mixture employed by Carroll and Richards was that of H. Steenbock and E. M. Nelson (*J. Biol. Chem.* **56,** *355 (1923)*), except that the calcium phosphate in the mixture was doubled. When this was compared with the basal diet containing the salt mixture devised by P. H. Phillips and E. B. Hart (*J. Biol. Chem.* **109,** *657 (1935)*), there was a decided decrease in digestibility of individual fatty acids, presumably because of the high concentration of calcium and phosphorus in the Phillips and Hart salt mixture. Another salt mixture, that proposed by R. B. Hubbell, L. B. Mendel and A. J. Wakeman (*J. Nutrition* **14,** *273 (1937)*), was compared with the Phillips and Hart mixture. A diet containing 2 per cent of the Hubbell-Mendel-Wakeman mixture yielded fatty acid digestion coefficients equal to those obtained with a diet containing 5 per cent of the Phillips and Hart mixture. When the former mixture was used at the 5 per cent level in the diet, the digestibility of oleic acid was reduced from 79 per cent to 42 per cent.

It is evident from these results that commonly used salt mixtures cover a wide range of calcium concentration and that the calcium content of the diet plays an important role in fat digestibility. The effect of other factors on the digestibility of erucic acid was also investigated. Digestibility appeared to be increased by a high-protein diet and decreased by a low-protein diet. Desiccated thyroid or thiouracil had relatively little effect although the thyroid-containing diet resulted in a marked increase in total food and food fat ingested. The digestibility of erucic acid appeared to be somewhat lower in old rats than in young growing rats (39 per cent vs. 53 per cent).

Another paper of interest relates to the effect of wool-fat sterols on the digestibility or absorbability of cholesterol in the rat (C. H. Duncan and M. M. Best, *J. Nutrition* **64,** *425 (1958)*).

The major components of wool-fat sterols, or "isocholesterol," are lanosterol, dihydrolanosterol, agnosterol, and dihydroagnosterol. Duncan and Best found that when lanosterol, dihydrolanosterol or agnosterol were included in the diet of rats at a 2 per cent concentration, they all exerted some effect in preventing the accumulation of cholesterol in the liver caused by the inclusion of 1 per cent dietary cholesterol. Dihydrolanosterol was most effective, followed by agnosterol and lanosterol. No absorption of lanosterol or dihydrolanosterol was detected, but small amounts of agnosterol were detected in the serum and livers of rats to which it was fed.

"Isocholesterol", when added to the diet at a 5 per cent concentration, was previously reported to inhibit the accumulation of cholesterol in the livers of cholesterol-fed rats (M. M. Best and C. H. Duncan, *Circulation Res.* **5,** *401 (1957)*).

Another study of interest is concerned with the effects of altitude and geographical conditions on serum cholesterol levels in human subjects (I. R. Payne, *J. Nutrition* **64,** *433 (1958)*). Data were obtained on

dietary, physical, and blood factors of 70 healthy women over 60 years of age who had lived at an altitude of 7200 feet (Wyoming) for at least two years. The data were compared with similar data on healthy persons of similar ages who had resided at sea level in California (H. L. Gillum and A. F. Morgan, *J. Nutrition* **55,** *265 (1955)*).

It was found that the total mean value for serum cholesterol was higher in the Wyoming women than in the California women. The women in the Wyoming study consumed a larger percentage of total fat in the form of animal fat and a greater percentage of the Wyoming women were over-weight.

From the results of these studies, it was concluded that altitude had a definite effect upon the serum cholesterol level. The theory is proposed that increases in hemoglobin, coinciding with increases in red blood cell counts, which occur at high altitude, are accompanied by an increase in degenerate "ghost" cells which contribute cholesterol to the serum. As an associated hypothesis it is proposed that the increase in serum cholesterol which occurs with increase in age in human subjects may be due to hematopoietic changes which occur with age, and which resemble those changes found in persons residing at high altitude.

This is certainly an interesting theory and perhaps deserves the serious attention of investigators in the field of lipid metabolism and nutrition. The partial correlation coefficient (0.393) of hemoglobin to total serum cholesterol that was found in this study is in support of the theory proposed, although, based on the data presented, it in no way directly implies any cause-effect relationship.

IN VIVO PROTEIN DIGESTION

Many factors which affect protein digestion and the absorption of the products of digestion in the intact animal are, at present, not clearly understood. There is, for example, no ready explanation for the differences in rate of protein hydrolysis *in vitro* versus *in vivo*.

The rate at which a protein is hydrolyzed as it passes through the digestive tract may or may not have an influence upon its ultimate value (O. Mellander, *Nutrition Reviews* **13,** *161 (1955)*). When human or cow's milk was fed to infants, for example, the degree of hydrolysis of protein in the stomach, as determined by the Van Slyke analysis for amino nitrogen, was found to be greater for cow's milk than for human milk.

It is nonetheless important to determine the rates of digestion of various proteins in the intact animal in order to determine whether or not the overall digestibility or nutritive value of a protein is in any way related to its ease of digestion.

Workers at the University of Wisconsin, Madison, have recently obtained information on the relative rates of disappearance of protein (nitrogen) from the digestive tract of rats (J. D. Gupta, A. M. Dakroury, and A. E. Harper, *J. Nutrition* **64,** *447 (1958)*).

Adult male rats were trained to consume the daily ration during a single two-hour period each day. Animals subjected to such treatment suffered weight loss initially but usually were conditioned, and had regained their starting weights, in two weeks.

The basal diet used consisted of the following: test protein, 15; salts, 4; corn oil, 5; vitamin supplement, 0.4; and sucrose to make 100 per cent. The proteins studied were defatted beef, alcohol-extracted casein, and zein. A protein-free diet was also used to establish endogenous nitrogen levels in the digestive tract. In one diet the test protein was replaced by a mixture of indispensable amino acids equivalent to 1.25 times the requirement. The inclusion of 1 per cent

of glycine and 3.5 per cent of glutamic acid in this diet brought its nitrogen content to the same level as in the test protein diet.

Rats trained in the above manner were offered 6 g. of the diets. Data were obtained only on the animals that consumed 4 to 5 g. of diet in 10 to 15 minutes. The animals were sacrificed at regular intervals after feeding. Contents of the stomach and small intestine were analyzed for nitrogen as a measure of rate of disappearance of dietary protein. Nitrogen thus measured is recognized to be influenced by both the rate of digestion and the rate of absorption of the products of digestion.

With this procedure it was established that beef proteins, casein, zein, and amino acid mixture passed from the stomach at about equal rates during the first two hours after feeding. During this period about 60 per cent of the ingested nitrogen disappeared. After two hours zein disappeared less rapidly than the other nitrogen source, and at the end of six hours it was the only nitrogen source present in excess of 20 per cent of the amount ingested.

The data obtained from the small intestine contents indicated that protein feeding raised nitrogen levels in this region of the digestive tract only slightly above the endogenous level, with the exception of the alcohol-soluble protein, zein. Considering nitrogen recovery from the entire digestive tract (stomach and small intestine) six hours after feeding, the data reveal approximately a 50 per cent recovery for zein and approximately a 20 per cent recovery for the remaining nitrogen sources. The data clearly demonstrate the slower disappearance of the alcohol-soluble protein, zein.

The rate of disappearance of nitrogen from the stomach was found to agree well with the stomach-emptying time in rats as observed in other studies (S. Lepkovsky *et al.*, *Am. J. Physiol.* **188,** *327 (1957)*; A. E. Harper and H. E. Spivey. *Fed. Proc.* **16,** *387 (1957)*).

In comparing the results obtained by Gupta and associates (*loc. cit.*) with those of other workers, it is evident that other dietary constituents consumed with the protein may influence its disappearance. Thus, the present paper reports that on the complete diet 40 per cent of ingested zein was still present in the stomach four hours after feeding, whereas an earlier report with this protein indicated that 60 per cent of the test dose fed was found in the intestine within two hours after feeding (E. Gieger, *Fed. Proc.* **10,** *670 (1951)*). Gieger noted that mixing the protein with carbohydrate or fat slowed both stomach-emptying time and the rate of disappearance of dietary nitrogen.

The results of L. S. Dreisbach and E. S. Nasset (*J. Nutrition* **53,** *523 (1954)*) had shown earlier that the type of dietary carbohydrate affects the rate of disappearance of protein from the digestive tract; more complex carbohydrates caused the rate to be slower.

The observation that free amino acids did not disappear from the digestive tract more rapidly than intact proteins (casein or beef protein) suggests that the rate of digestion is not a limiting factor in protein utilization unless the protein is relatively insoluble (zein).

The limited data available on the passage of dietary protein, or its digestive products, suggest that a number of dietary factors may have an influence on the rate of passage. The effects of total food intake, the effects of various dietary components, and, possibly, the effect of age of the intact animal should be considered as important areas for investigation.

It would also seem important to find a means of separating dietary protein more accurately from other protein sources in the digestive tract before reliable conclusions can be drawn. In the studies by Gupta and associates there was some indication,

for example, that beef protein may have stimulated secretory activity in the digestive tract, as evidenced by more than 100 per cent recovery of nitrogen at zero time after feeding. Dreisbach and Nasset (*Loc. cit.*) were able to recover more nitrogen from the digestive tract than was fed as late as five hours after feeding, when only small amounts of protein were administered. This would indicate that the contribution of nitrogen from digestive enzymes to the contents of the digestive tract can be considerable and can magnify the error in such measurements considerably, particularly if relatively low levels of test protein are administered. In the studies by Gupta and associates endogenous nitrogen in the digestive tract represented only about 10 per cent of the total nitrogen present immediately after the test diets were fed.

ALLITHIAMINE

In the early 1950's, in a search for antithiamine factors, M. Fujiwara, H. Watanabe and K. Matsui (*J. Biochem.* **41,** *29 (1954)*) isolated from allium sativum (garlic) an active principle which was capable of converting thiamine to a compound which gave a negative thiochrome test but showed vitamin B_1 activity *in vivo*. This action was termed by the authors a "masking action" rather than an inactivation. Further study indicated that the new compound, called allithiamine, was formed from thiamine by the 3000 r.p.m. supernatant solution of an aqueous extract of fresh garlic bulbs. The optimum conditions for the reaction were 70° and pH 8. Since the boiled extract was inactive, an enzyme action was indicated. Investigation along these lines revealed that the enzyme alliinase catalyzes the conversion of alliin (S-allylhomocysteine sulfoxide) to allicin (diallyl disulfide, monosulfoxide). This reaction could easily be followed by the production of pyruvic acid and ammonia when an extract of the garlic-like plant Ipheion uniforum was incubated with the garlic extract. The allicin or allicin homologue formed in this reaction reacts with thiamine thiol to form the allithiamine (in which the thiol is involved in a disulfide linkage).

In an investigation of alliin homologues in other plants of the allium family (liliaceae) and brassica family (cruciferae), Fujiwara, M. Yoshimura, S. Tsuno and F. Murakami (*J. Biochem.* **45,** *141 (1958)*), found that the enzyme, alliinase, is present only in plants of the allium species. Paper chromatographic analysis, however, revealed that various homologues of alliin, in which the allyl group was replaced by methyl, ethyl or propyl, were present in different proportions in various members of the allium and brassica species, the methyl alliin being by far the most prevalent type. Action of the enzyme on any of these alliin homologues converted them to the corresponding allicin compounds, which could react with thiamine to give the various allithiamines. This action was found to occur not only in vitro, but also when plants containing alliinase and an alliin homologue were eaten simultaneously.

The results of the pharmacological and nutritional studies carried out on allithiamine were reported by Fujiwara, H. Nanjo, T. Arai and S.-Ziro. (*J. Biochem.* **41,** *273 (1954)*). Toxicity tests in mice revealed that while allithiamine was about one third as toxic as thiamine intravenously, it was considerably more toxic when administered orally. The authors considered this as evidence that allithiamine is much more readily absorbed from the intestine than is thiamine. This assumption was borne out by further studies. Rats on a thiamine-deficient diet plus 4.2 micrograms per day of allithiamine grew about 20 per cent faster than those with an equivalent thiamine supplement.

Allithiamine was also about as effective as thiamine in curing bradycardia in rats or convulsions in pigeons on thiamine-deficient diets.

In human experiments, a much higher urinary excretion and a lower fecal excretion of thiamine were obtained after oral doses of allithiamine than was found with thiamine itself, again indicating a very rapid and complete absorption of the allithiamine. Studies of blood thiamine levels in rabbits showed that higher levels were obtained in plasma after injection of allithiamine than with thiamine. In this case, a much greater incorporation into the erythrocytes was found and may have indicated a greater absorption by these cells as well as those of the intestinal wall. Indeed, several other organs, such as liver, small intestine and kidney had higher thiamine levels after injection of allithiamine than after thiamine itself.

The authors point out that these studies may provide a means of rapidly increasing blood thiamine levels with a minimum of inconvenience to the physician and discomfort to the patient. Indeed, it appears from these studies that the much maligned garlic may have a therapeutic value.

PROTEINURIA IN RATS AND MICE

It has been recognized for many years that both rats and mice excrete considerable amounts of protein in their urine. About 30 years ago, there was appreciable activity in this field, but then, as happens with so many scientific discoveries, the earlier observations were largely forgotten. It is likely that the original work was terminated because as much progress had been made as was possible with existing techniques. During the past few years, there has been a resurgence of activity in this field. The more recent work has covered not only the protein excretion of normal rats and mice but the factors that influence the level of urinary protein excretion.

Much of the earlier work on this problem was done by T. Addis, E. M. MacKay and L. L. MacKay at Stanford University (*J. Biol. Chem.* **71,** *139 (1926)*) who placed weanling rats on semi-purified diets containing either 16 or 74 per cent casein. They first collected 24-hour urine samples from their animals on the two-hundred-seventieth day of the experiment and found that the male rats were excreting from 2 to 15 mg. of protein per rat per day, whereas the females excreted 0.2 to 4.0 mg. For both the males and the females, the excretion of protein was higher in the group receiving the 74 per cent casein diet.

As an extension of this work, Addis (*Proc. Calif. Academy of Medicine* **2,** *38 (1932)*) castrated weanling male rats. The castrated animals showed a proteinuria which was intermediate between that of the males and females. He also found that the proteinuria in weanling male rats was the same as that in females. However, the proteinuria in male rats rapidly increased from a level of approximately 1.4 mg. of protein per 24 hours per 100 sq. cm. of body surface to 7.0 mg. by the hundredth day of age, after which it decreased to 4.7. In the females there was no essential change with age. To rule out the accessory sex organs in the male as the source of the urinary protein, Addis (*Loc. cit.*) removed the vasa differentia and the seminal vesicles. Neither of these operations had any effect on the proteinuria.

Work in this field was relatively dormant for almost 20 years. At the end of that time, A. L. Sellers and co-workers (*Am. J. Physiol.* **163,** *662 (1950)*) re-investigated the influence of castration on the proteinuria of rats. They found that when weanling male rats were castrated, the proteinuria increased slightly, as reported by Addis (*Loc.*

cit.). However, by the one-hundred-fiftieth day of the experiment, the proteinuria of castrated male rats was down to that of the females, whereas the proteinuria of the normal male rats continued at its usual high level. In confirmation of Addis' observation, they also found that castration of adult male rats reduced the proteinuria to a level which was approximately half-way between that of the normal male and female. Ovariectomized females showed no change in proteinuria, whether the operation was done on weanling or adult rats. Sellers, *et al.*, (*Loc. cit.*) suggested that in the adult male rat the kidney had been under the influence of testicular hormones long enough to produce irreversible changes in the kidney leading to a high level of proteinuria. However, in the weanling animal the action of the testicular hormone had been of such short duration that the kidney had not been irreversibly influenced; consequently castration produced a marked reduction in proteinuria.

That the action of the testes was due to the presence of testosterone was shown by the above workers (Sellers *et al.*, *Loc. cit.*) who found that the injection of this hormone into castrated male and female rats produced a prompt increase in urinary protein excretion. At the termination of the injections, the proteinuria returned to its pre-injection level within 25 to 50 days.

Sellers and co-workers (*Loc. cit.*) found that the protein content of urine removed directly from the bladder was the same as that found in the voided urine. This suggested that the other organs in the genito-urinary system contributed practically no protein to the urine and that the proteinuria was very likely of renal origin.

The proteins present in the urine were shown by electrophoretic analysis to consist of two major components (Sellers, *et al.*, *J. Exp. Med.* **95,** *465 (1952)*). The major fraction was similar to serum α_2 globulin. The minor component had a mobility midway between that of α_1 globulin and albumin. Ammonium sulphate precipitation of the proteins in the urine added confirmatory evidence for the above and suggested the presence of small amounts of albumin in rat urine. These investigators proposed that the proteinuria was due to the permeability of the glomerular membrane to protein. Since the globulins have a molecular weight which is considerably greater than that of albumin, the latter protein should also pass the glomerular membrane. This, Sellers and co-workers (*Loc. cit.*) suggests, happens, but the albumin is absorbed in the tubules.

The effect of the high protein diet in increasing the proteinuria of rats led J. S. Finlayson and C. A. Baumann at the University of Wisconsin (*Am. J. Physiol.* **184,** *469 (1956)*) to study the influence of dietary urea on the phenomenon. They used a purified diet containing 12 per cent casein, 4 per cent Wesson salts, 5 per cent corn oil, 0.2 per cent cystine, and glucose to make 100 per cent; vitamins in adequate amounts were added. When urea was added to this diet at the expense of glucose, proteinuria increased with increasing levels of dietary urea up to 20 per cent. Beyond that level, urea had no further influence on the amount of protein excreted. Blood urea nitrogen levels showed a linear relation to dietary urea, even up to levels of 30 per cent. The latter observation would suggest that the blood urea level alone was not the primary cause of the proteinuria.

Finlayson and Baumann (*Loc. cit.*) compared the effect of urea and an isonitrogenous amount of casein added at the expense of glucose to the 12 per cent casein diet. They found that the added casein produced considerably more urinary protein than the urea. In these experiments, the blood urea nitrogen was much higher when urea was present in the diet than when additional casein was present.

To minimize the contamination of the urine with dietary protein, Finlayson and Baumann (*Loc. cit.*) trained their animals to eat their daily food within a period of two hours. Under these circumstances, they

found that after starting the experimental diet the proteinuria in the rats receiving the urea in the diet increased to its maximum level within a period of four hours. The animals receiving the casein diet attained maximum levels of proteinuria ten hours after the diet was started.

When the influence of an additional 20 per cent casein added to the diet was compared to that of urea or an amino acid mixture equivalent on a nitrogen basis to that of the added casein, it was observed that the proteinuria in the rats receiving the amino acid diet was the same as that in the group receiving the urea diet. The proteinuria in the rats receiving the added casein was approximately twice that of the amino acid group (Finlayson and Bauman (*Loc. cit.*)).

Forcing the rats to drink more water (the procedure used was not stated) had no influence on the proteinuria of the animals fed the purified diet regardless of the level of dietary protein. However, on a stock diet, increased water intake increased proteinuria, while an urea solution equal in volume to that of the water had no additional effect on the proteinuria beyond that exhibited by the water (Finlayson and Baumann, *Loc. cit.*).

Similar results were secured by H. W. Rumsfeld, Jr. (*Am. J. Physiol.* **184,** *473 (1956)*) who studied the influence of urea on the proteinuria of male rats. In order to avoid contamination of urine with food, Rumsfeld fasted his rats for 48 hours. Under these circumstances, the animals receiving the added urea or added casein showed the same amount of proteinuria as the animals on the basal (18 per cent casein) diet. A 24-hour fast resulted in a slightly higher level of proteinuria in the rats receiving added casein or urea when compared with the basal group. These observations suggest that some phenomenon associated with the actual metabolism of dietary protein (this might well be the quantity of protein presented to the tissue for metabolism) was the important factor influencing the level of proteinuria.

Proteinuria in mice has been recognized as a normal occurrence since the observation of I. A. Parfentjev and W. A. Perlzweig (*J. Biol. Chem.* **100,** *551 (1933)*). They found that when mice were maintained on a diet of whole wheat, oats, and bread, they excreted urine containing an average of 1.5 per cent protein (range 1.3 to 2.1). Limited physical measurements suggested that mice excreted a chondromucoid protein. They were impressed by the concentrated urine voided by mice. The specific gravity of mouse urine ranged from 1.045 to 1.064, with an average of 1.058. This is considerably greater than the specific gravity of human urine (1.015 to 1.025).

L. F. Wicks (*Proc. Soc. Exp. Biol. Med.* **48,** *395 (1941)*) showed that the proteinuria in mice was a characteristic of all the strains he studied. Here, as in rats, the proteinuria was much greater in the males than in the females. He also showed that bladder urine contained as much protein as voided urine. His mice showed an increased proteinuria when they received a diet containing 33 per cent casein compared to one containing 5 per cent casein. Castration of the mice produced a marked reduction in the proteinuria three months after the operation.

Finlayson and Baumann (*Am. J. Physiol.* **192,** *69 (1958)*) compared the proteinuria in rats and mice. When expressed in terms of body weight, the urinary protein excretion of male mice was much greater than that of rats (0.18 to 0.53 mg. protein nitrogen per 100 g. of rat versus 3.1 to 7.5 mg. for mice). The urinary excretion of protein by male mice was much greater than that of females (3.1 to 7.5 mg. protein nitrogen versus 0.15 to 1.7 for females). Electrophoretic analysis showed that the proteins excreted by the mice were the same regardless of the strain of animal used. Unlike the rat, the major urinary protein excreted by the mouse is something other than serum albumin or globulin.

The protein excreted by both rats and mice has bound to it both cholesterol and

another sterol which is presumably Δ^7cholestenol (Finlayson and Baumann, *Am. J. Physiol.* **190,** *297 (1957)*). Normal adult male rats excreted an average of 15 gamma of cholesterol in the urine per day and 2 gamma of cholestenol. The sterols in rat urine were bound to both albumin and globulin (about 75 per cent cholesterol was bound to the globulin, the remainder to the albumin; about 60 per cent of the cholestenol was bound to the globulin, the remainder to the albumin). Mice showed considerable strain difference in the amounts of sterols in the urine, with the Rockland mice excreting 3.0 gamma cholesterol per day and 0.3 gamma Δ^7cholestenol, while the Swiss-Webster mice excreted 0.7 gamma and 0.1 gamma, respectively. Most or all of these sterols are presumably bound to the urinary protein.

The presence of protein in human urine as measured by the acetic acid-heat test (described by Dekker in 1694 according to Addis, *Calif. Academy of Medicine* **2,** *38 (1932)*) suggests the presence of a disturbance or disease of the kidney. More sensitive tests for protein indicate that small amounts of protein occur in the urine from normal individuals (Addis, *Loc. cit.*). By the application of special methods, 24-hour urinary excretion of protein by normal men was found to be 0.01 g. (Addis, *Transactions of the Assoc. of Am. Physicians* **57,** *106 (1942)*).

The recent work on the urinary excretion of proteins by rats suggests very strongly that these animals routinely excrete small fractions of certain serum proteins. The characterization of the protein excreted by the mouse remains to be clarified. The excretion of these proteins by the kidneys is influenced to a considerable extent by testosterone. The action of testosterone on the kidneys is partly irreversible, as shown by the partial reduction in urinary protein levels when castration is performed in adult rats, whereas castration of weanling rats bring the level down to that of females. Injection of testosterone into castrated male or normal female rats increases the degree of proteinuria.

The elucidation of the mechanisms whereby the protein is excreted in rat and mouse urine should provide interesting and exciting research work for future investigators.

HYDROXYLYSINE IN COLLAGEN BIOSYNTHESIS

The knowledge that hydroxylysine and hydroxyproline are found only in collagen has stimulated interest in the origin of these two amino acids and in their role in determining the properties of this protein. Evidence has been presented in support of the concept that the conversion of proline to hydroxyproline occurs after the incorporation of proline into a non-hydroxylated collagen precursor. These considerations have stimulated K. A. Piez and R. C. Likins (*J. Biol. Chem.* **229,** *101 (1957)*) to study the origin of hydroxylysine in collagen.

The general approach of these investigators was to inject lysine labeled with carbon-14 into rats, together with a large amount of unlabeled hydroxylysine. After the various time intervals, the specific activities of the two amino acids in the collagen isolated from the experimental animals were compared with values obtained when animals were similarly treated but were not fed unlabeled hydroxylysine.

In the first experiment, five 50 g. male rats were injected with 60 mg. of hydroxylysine (an initial injection of 15 mg. in one ml. of water and 9 mg., similarly injected, each hour thereafter for five hours). Fifteen minutes after the first injection of hydroxylysine, 9 microcuries of uniformly C_{14}-labeled L-lysine hydrochloride were injected. A

control group received only the labeled lysine without any hydroxylysine. The rats were killed one hour after the last injection of hydroxylysine. In the second experiment, nine rats were injected with C_{14}-labeled lysine only. Three were killed after one hour, three after six hours, and the remaining three were killed after four days.

Gelatin was prepared from skin, tail, tendon, bones and dentin. The gelatin samples were hydrolyzed with acid. The amino acids were separated using ion-exchange columns. The amounts of lysine, hydroxylysine, proline and hydroxyproline in the hydrolysates were estimated and the amounts of radioactivity in lysine and hydroxylysine were determined.

In the first experiment, in which one group of rats was injected with C_{14}-labeled lysine along with a large amount of unlabeled hydroxylysine while the other group was injected only with the labeled lysine, serum lysine from both groups had the same specific activity. No hydroxylysine could be detected in sera from rats injected only with lysine. A very small amount of radioactivity was found in the serum hydroxylysine from the group injected with excess hydroxylysine.

The radioactivity measurements on the lysine and hydroxylysine from the various gelatin preparations obtained in this experiment indicated that the specific activity of hydroxylysine in the collagens was not decreased when excess unlabeled hydroxylysine was injected. The specific activity of the hydroxylysine in these preparations, made six hours after the injection of labeled lysine, was in most samples a little lower than that of lysine. The ratio of the specific activity of lysine to that of hydroxylysine varied from 1.0 for bone collagen to almost 1.4 for skin collagen. The ratio of the amount of lysine to that of hydroxylysine was different for each of the different collagens, being 1.1 for dentin, 1.9 for bone, close to 4.0 for tail tendon and about 6.0 for skin. The ratio for proline to hydroxyproline was about 0.8 for each of the samples. It, therefore, appeared that the differences in the lysine to hydroxylysine ratios represented real differences. In spite of the differences in the ratios, the total amount of lysine plus hydroxylysine was close to the same in each case.

In the second experiment in which the specific activities of lysine and hydroxylysine in skin collagen were measured at intervals after the injection of C_{24}-labeled lysine, the ratio of the specific activity of lysine to that of hydroxylysine fell with time. One hour after injection, the ratio was almost 1.4. This was unchanged after six hours, but after four days the ratio had fallen to one.

The results indicate that hydroxylysine injected into the rat is not incorporated directly into collagen even when the plasma level of hydroxylysine is high, a finding that parallels the earlier observations with proline and hydroxyproline and confirms observations by Sinex and Van Slyke (*Fed. Proc.* **16,** *250 (1957)*) that dietary hydroxylysine is not incorporated into collagen.

The results of the time study indicate that the specific activity of lysine was higher than that of hydroxylysine a short time after the injection of C_{14}-labeled lysine. This suggests that lysine is incorporated, as such, into the collagen molecule and that the hydroxylation reaction occurs after incorporation. The observation that the total lysine plus hydroxylysine was about the same for each of the collagens studied, whereas the ratios for lysine to hydroxylysine were different, also suggests that lysine is incorporated into the peptide chain and that conversion to hydroxylysine occurs later. It would also appear from these results that the hydroxylation reaction occurs at different rates in different tissues.

This work provides further evidence that dietary hydroxylysine is not incorporated into protein and supports the previous evidence that a non-hydroxylated precursor containing proline and lysine is formed before the hydroxylation reactions occur. Ascorbic

acid has been implicated in the hydroxylation of proline; one is tempted to speculate that it is also involved in the hydroxylation of lysine. Experiments in which the lysine and hydroxylysine content of the collagen precursors isolated from wound tissue from vitamin C-deficient guinea pigs would provide evidence on this point.

INTESTINAL PERMEABILITY TO PROTEINS

The transfer of immunity from mother to offspring has been a problem that has intrigued investigators for many years. Considerable evidence has accumulated suggesting that in some animal species, colostrum is the means whereby some of this immunity may be transferred. Evidence for this statement comes from the finding that there are proteins in bovine colostrum secreted immediately after parturition which resemble in many ways those that appear in the blood of the calf permitted to suckle its mother from birth. These so-called "immune" proteins disappear from the colostrum within two days after parturition. The question has recently been raised as to whether the disappearance of these "immune" proteins from the colostrum coincided with the loss of absorptive ability on the part of the calf for these proteins. Another problem that has aroused some interest is the search for the factors that decrease the absorptive capacity of the calf's intestinal tract for proteins.

The renowned Rockefeller Institute bacteriologist Theobald Smith, with R. B. Little, carried out a series of experiments which strongly suggested that practically all calves permitted to suckle their dams survived for one month or more while those receiving nothing but milk died with bacterial infection in all their organs (*J. Exp. Med.* **36,** *181* (*1922*)). The calves receiving no colostrum could be kept alive by an injection of serum from a lactating cow; better results were secured when this procedure was combined with the addition of serum to the milk fed the calves during the first day of life (*Ibid.* **36,** *453* (*1922*)).

This demonstration of the importance of colostrum in calf nutrition early raised the question as to whether a similar situation exists in human infants. Ann Kuttner and B. Ratner (*Am. J. Dis. Child.* **25,** *413* (*1923*)) showed that diphtheria antitoxin was transmitted from mother to infant through the placenta. The antitoxin titer of human colostrum was low but detectable. In spite of the presence of antibodies in the colostrum, there was no evidence that the infant's blood titer increased as a result of colostrum feeding.

Additional suggestive evidence for the non-transfer of "immune" proteins by means of human milk comes from the recent studies of H. J. B. Atkins (*Nutrition Reviews* **16,** *261* (*1958*)). He reported that there was no difference in the incidence of gastroenteritis, broncho-pneumonia, otitis media, rubella, pertussis, and a number of other infections among children who never received any human milk and those who received their mothers' milk (whether this included colostrum is not apparent from the paper).

The explanation for the difference in the reaction of infants and calves to the proteins in colostrum resides in the thickness of placental barrier in the two species. The membrane separating the maternal from the fetal blood is one cell thick in the human, guinea pig, rat and rabbit; it is permeable to a variety of proteins. In the cow, goat and sheep, the placental barrier is three cell layers thick and consequently is more impermeable to proteins (B. Ratner, H. C. Jackson and H. L. Gruehl, *J. Immunol.* **14,** *249* (*1927*)).

Significant changes occurred in the composition of the blood proteins of calves following the feeding of colostrum. P. E. Howe (*J. Biol. Chem.* **49,** *115* (*1921*)) used his technique of precipitating different proteins with various salt solutions to show that at birth the calf has neither euglobulin nor pseudoglobulin I in its blood. Within two days after birth, these proteins increase very rapidly in the blood when colostrum is fed.

I. I. Earle (*J. Agric. Res.* **51,** *479* (*1935*)) extended the above work to foals, kids, lambs and pigs. She found that the animals receiving no colostrum from their dams showed little or no increase in serum globulin levels. Those animals that did receive colostrum showed an increase in serum globulin, with the greatest increase occurring in the second day of life, followed by a slow decrease during the next fourteen days (*e.g.*, one hour, one, three, six, and thirteen days following delivery the euglobulin blood levels in a kid were 0.006, 0.21, 0.14, 0.17, 0.10 g. of nitrogen per 100 ml. of blood, and the pseudoglobulin levels were 0.09, 0.23, 0.16, 0.16, 0.14 g. of nitrogen per 100 ml. blood, respectively).

Electrophoretic analysis of the isolated proteins (E. L. Smith, *J. Biol. Chem.* **164,** *345* (*1946*)) showed that the immune globulin isolated from colostrum was similar to the T compound of calves' blood (this fraction is the same as the pseudoglobulin of the earlier workers). Although the colostral and blood proteins had the same physical constants and anaphylatic reactions (as measured in guinea pigs), they differed in amino acid and carbohydrate composition (E. L. Smith, R. D. Greene and E. Bartner, *Ibid.* **164,** *359* (*1946*)) as well as in ultra-violet absorption spectra (E. L. Smith and N. H. Coy, *Ibid.* **164,** *367* (*1946*)). The latter findings suggested that there was a slight change in the colostral proteins associated with their absorption and transfer to the blood stream of the calf.

In the colostrum secured immediately post-partum, the "immune" globulin represented 55 per cent of the total protein (E. L. Smith, *Ibid.* **164,** *345* (*1946*)). The level of this protein in the colostrum decreased rapidly so that by the second day there was very little, if any, present.

The mechanisms whereby the proteins are transferred from the colostrum to blood was studied by R. G. Hansen and P. H. Phillips (*J. Biol. Chem.* **179,** *523* (*1949*)). They could distinguish electrophoretically between the immune globulins in the colostrum from cows, pigs and goats. When new-born kids were fed the first postpartum sample of colostrum from a cow, the protein appearing in the blood of the kid seemed to be the same as that in the colostrum. In other words, the kids appeared to absorb the "immune" proteins of cows' colostrum. Contrary to the conclusion arrived at by Smith (*loc cit.*), Hansen and Phillips proposed that there was no alteration in the proteins as a result of intestinal absorption. They reported, however, that there is a certain amount of selectivity in the absorption of proteins by the new-born kid as shown by the fact that other colostral and milk proteins fail to appear in the blood.

Immunological studies by Hansen and Phillips (*loc. cit.*) showed the presence in blood from a new-born calf, prior to feeding, of a protein similar to cow colostral pseudoglobulin (immune globulin). This finding is unusual since most of the previous investigators claimed that specific antibodies did not appear in calf serum until after colostrum from a newly freshened cow was fed. They also found by the immunological technique that feeding milk to new-born calves increases the "immune" protein level of the blood. The increase was so slight that it could not be detected by electrophoretic analysis of calf serum (Hansen and Phillips, *Ibid.* **171,** *223* (*1947*)). These findings can be interpreted on the basis of the relatively high sensitivity of the immunological assay technique compared with the electrophoritic procedure.

The fact that hervibora lose their ability to absorb immune proteins within 24 hours after birth suggested the possibility to H. F. Duetsch and V. R. Smith (*Am. J. Physiol.* **191,** *271* (*1951*)) that food or humoral factors were associated with this change. To test their hypothesis, they fed milk to new-born calves and gave them either diethylstilbestrol (100 to 300 mg.), progesterone (200 to 500 mg.), a combination of these two hormones, cortisone (200 to 350 mg.) or ACTH (120 I.U.) either intramuscularly, intraperitoneally or subcutaneously. None of these substances prolonged the period during which the intestinal mucosa was permeable to "immune" proteins.

There is no specific factor in the colostrum or milk which brings about the change in intestinal permeability. This was shown by feeding a semi-purified diet, with the amino acids (Aminosol) being given intravenously. A variety of other substances including pancreatic desoxyribonuclease, inhibitors of gastric activity (Probanthine), and transfusion of maternal blood into the new-born calf also had no effect on the decrease in intestinal permeability to proteins occurring in calves 24 hours after birth.

Although Deutsch and Smith (*loc. cit.*) were unsuccessful in their efforts to prolong the permeability of the calf's intestine to proteins, they were able to show that all colostral proteins were absorbed. Except for the "immune" proteins which accumulated in the blood, the major portion of the other colostral proteins were excreted in the calf's urine. This verifies the earlier observation of Howe (*loc cit.*) who reported that calves' urine contains large amounts of protein and that the proteinuria disappears in 24 to 48 hours after birth. It also explains the contention of Hansen and Phillips (*J. Biol. Chem.* **179,** *523* (*1949*)) that the absorption of colostral proteins was selective since they could find evidence for the accumulation in calves' blood of only the "immune" colostral proteins.

There are a number of unresolved problems associated with the above phenomena. Most of the earlier studies have been of a qualitative nature. It would be important to know what fraction of the ingested "immune" proteins appears in the blood and what fractions of the other proteins are excreted in the urine. Since the presence of the "immune" proteins in the colostrum ceases at the same time that the new-born loses its ability to absorb them, one might consider the possibility that the changes in the intestinal permeability of the calf and the secretory activity in the dam's udder are related.

The above studies show that the new-born calf, foal, lamb, kid and pig are able to absorb certain proteins from the colostrum and that these proteins then appear in the animal's blood. These proteins may play an important role in increasing the resistance of the animals to a variety of infections. There is a period of only one to two days following birth during which this absorption of proteins can occur. This is the same period during which the proteins appear in the dam's colostrum. Although colostral proteins other than those appearing in the globulin fraction of the blood are absorbed by the new-born animals, the other proteins are excreted in the urine.

THE AVAILABILITY OF LYSINE IN FOODS

It has been demonstrated on many occasions that the nutritive value of the protein in a food may be reduced by procedures involving heat (*Nutrition Reviews* **2,** *167* (*1944*); **3,** *238* (*1945*)). Much of the decrease in nutritive value has usually been made good by supplementation of the processed material with lysine, although analysis for lysine by conventional means has shown little change in its concentration. It has been

concluded, therefore, that the lysine has become in some way "bound" or "unavailable" (*Ibid.* **5,** *83 (1947)*; **8,** *193 (1950)*).

Several recent studies have aimed at discovering how far such an effect may be limiting the nutritive value of foods and animal feedstuffs as normally prepared. In particular, biological assays of the lysine available in foods to rats have been carried out with modifications of the procedures described by B. S. Guthneck, B. A. Bennett and B. S. Schweigert (*J. Nutrition* **49,** *289 (1953)*).

W. S. Tsien, E. L. Johnson and I. E. Liener (*Arch. Biochem. Biophys.* **71,** *414 (1957)*) have studied the availability of the lysine in a sample of *Torula* yeast. Microbiological assay with *Leuconostoc mesenteroides* P-60 of an acid hydrolysate gave a value of 4.1 per cent for the lysine content of the material. For the rat assays these workers used a basal diet containing a full amino acid mixture, with the exception of lysine, on which the young rats lost weight; with the addition to the diet of 0.9 per cent of lysine there was an average weight gain of 20 g. per week.

Different groups were then fed supplements of approximately 0.3, 0.5, 0.7 and 0.9 per cent of pure lysine and corresponding supplements of Torula yeast calculated to contribute the same levels of lysine. At each level of feeding the yeast, approximately 10 per cent of the calculated lysine intake was recovered in the feces, whereas the pure lysine was almost entirely absorbed. Despite this, the growth response was at each level greater with yeast than with pure lysine. The authors conclude, therefore, that the lysine in the material must have been largely available, although the presence of some other growth-stimulating factors prevented the assay from giving a straight-forward quantitative measure. Alternatively, of course, the values for lysine given by the usual type of microbiological assays could, for certain foods, be too low; although there seems to be no other evidence to support this hypothesis.

K. J. Carpenter (*Proc. 4th Internat. Congr. Nutr. Paris, 155 (1957)*) has reported the use of a basal diet for lysine assay with rats that is fortified with wheat gluten, dried skim milk and sesame seed meal as possible sources of growth factors not found in a "synthetic" type of ration. Such additions have the drawback, of course, of making the basal ration less deficient in lysine and thus allowing only a smaller range of response to supplements. However even with these additions a high-quality protein food gave a greater response than was to be explained by its content of lysine as determined by acid-hydrolysis and microbiological assay.

Most recently J. D. Gupta, A. M. Dakroury, A. E. Harper and C. A. Elvehjem (*J. Nutrition* **64,** *259 (1958)*) have reported more assays with a basal diet containing 20 per cent of wheat gluten together with a range of amino-acids, and having dextrin as the main energy source. From the results obtained it was concluded that "weight gain per weight of food eaten" was a truer measure of response than "rate of weight gain" by itself. However, even with this procedure one material, a soyabean preparation, gave a figure of 118 per cent availability against the lysine figure obtained by microbiological assay. A range of animal protein foods gave figures ranging from 83 to 104 per cent for the availability of their lysine. The corresponding mean figures for corn, wheat flour and rice were 54, 71 and 87 per cent, respectively.

B. P. Baliga and C. M. Lyman (*J. Am. Oil Chem. Soc.* **34,** *21 (1957)*) have reported further studies on the evaluation of cottonseed meals for rats by taking the level of lysine recoverable in their feces as a measure of its unavailability. However the special problem of the interaction of gossypol and lysine in cottonseed proteins cannot be considered here.

Generally similar assays carried out with

young growing chickens have also been reported. W. S. Tsien, *et al.*, (*loc. cit.*) conclude that the lysine of *Torula* yeast was very largely available for chicks, as it had been for rats. However, the comparison of live-weight gains with nitrogen retention data indicated a complicating factor. Although the response to the yeast in terms of feed utilization and total nitrogen retention were both higher than could be accounted for by its lysine content, the nitrogen retention calculated on a "per unit body weight" basis was less. This finding is difficult to interpret.

F. H. Kratzer and N. Green (*Poultry Sci.* **36,** *562 (1957)*) have used chicks to estimate the availability of the lysine in commercial samples of blood meals. The basal ration contained 20 per cent of protein which came from sesame seed meal and corn with no further addition of synthetic amino acids. The blood meals were added to the experimental diets at the expense of sesame seed meal and corn starch so as to keep the total protein level constant. Simple body weight gain for a 10 to 12 day period was taken as the measure of response; and, as usual, the results were compared with lysine values obtained by microbiological assay of the blood meals. It was concluded that for three samples prepared by careful spray-drying at 145° F, the mean availability of the lysine for chicks was 75 per cent, while for two vat-dried meals it was 65 per cent.

J. Mauron (*Rev. Internat. Vitamin*, **27,** *85 (1956)*; *Proc. 4th Internat. Congr. Nutr. Paris, 159 (1957)*) has used rat assays as a standard to investigate the significance of analytical values for dried milk obtained from estimation of the lysine released by an *in vitro* enzymic digestion described earlier by J. Mauron, F. Mottu, E. Bujard and R. H. Egli (*Arch. Biochem. Biophys.* **59,** *433 (1955)*). The results confirm that with drastic heat treatment the reduction in the proportion of lysine that is released by enzyme treatment is much greater than the actual destruction of lysine, and that it is the former measure which more closely parallels the results of rat assays.

In view of the inadequacy of those chemical or microbiological procedures that begin with an acid-hydrolysis for the estimation of lysine, K. J. Carpenter, G. M. Ellinger, M. I. Munro and E. J. Rolfe (*Brit. J. Nutrition* **11,** *162 (1957)*) have proposed a different approach which has been applied to animal protein foods, or in a modified form to all types of protein-containing foods (D. Bruno and K. J. Carpenter, *Biochem. J.* **67,** *13 (1957)*). The procedure exploits the reaction of fluorodinitrobenzene (FDNB) with the free amino-groups of intact proteins, the reaction that proved so profitable in the determination of the structure of insulin by F. Sanger (*Biochem. J.* **45,** *563 (1949)*).

Those lysine units in the intact food that have their ϵ-NH_2 groups free react with FDNB during a period of digestion, and in a subsequent period of hydrolysis ϵ-dinitrophenyl lysine is released and can be measured colorimetrically after a simple separation of those interfering materials that are likely to be present in significant quantities. Where the ϵ-NH_2 groups are already blocked, by the Maillard browning reaction or otherwise, no ϵ-DNP lysine is formed. Reaction through the ϵ-NH_2 group, with linkages resistant to the digestive enzymes, has been generally considered as the most likely explanation of the vulnerability of lysine to damage in food processing (*Nutrition Reviews* **5,** *83 (1947)*).

In the tests of the FDNB procedure on a range of foodstuffs the values obtained have shown a good correlation with the results of standardised chick feeding tests where the materials were fed as supplements to a basically cereal diet in which lysine was the first (but not the only) limiting amino-acid (Carpenter, *et al, loc. cit.*; Carpenter, *Proc. Nut. Soc.* **17,** *91 (1958)*). The materials tested included dehydrated cod that had been subjected to different degrees of heat treatment. These treatments caused a

greater reduction in the lysine as assayed by the FDNB procedure than by the usual microbiological assay. The FDNB procedure has also given values for a short series of six animal protein samples (of which three had been heat-damaged) that correlated more closely with the results of rat assays than did values obtained by microbiological assay. Obviously biological assays must remain the method of choice but it seems that the modified chemical method may prove useful as an indicator of damage in circumstances where a biological test is impracticable.

CHOLINE DEFICIENCY AND THE MORPHOLOGIC EFFECTS OF SEROTONIN

Interest in the chronic toxicity and morphologic effects of serotonin comes partly from the fact that serotonin (5-hydroxytryptamine) is found in carcinoid tumors (F. Lembeck, *Nature* **172,** *910 (1953)*). Patients having a metastasizing carcinoid tumor of the gastrointestinal tract experience flushing, cyanosis, dyspnea, diarrhea, and endocardial valvular lesions of the right heart. They also show an increased incidence of peptic ulcer. In addition, these patients excrete large quantities of 5-hydroxyindoleacetic acid, a product of serotonin degradation. Experimental animals injected with serotonin show the same clinical symptoms associated with the human syndrome but morphologic effects have not been evaluated because of the difficulty in maintaining life of the animals.

R. A. McDonald, S. L. Robbins and G. K. Mallory (*Arch. Path.* **65,** *369 (1958)*) undertook a study of the morphologic effects of serotonin creatinine sulfate in rats, especially with regard to the cardiovascular changes. Rats proved to be especially suitable because of their susceptibility to the drug and because subcutaneous injection was most effective and technically uncomplicated.

Human carcinoid patients with valvular lesions often show fatty changes of the liver. Furthermore, the presence of the tumor in the livers of most of the patients developing the carcinoid syndrome suggests that the liver may play an important role in the inactivation of serotonin. Accordingly, some of the serotonin-treated animals were fed choline-deficient, high-calorie, high-fat diets which produced fatty livers leading to cirrhosis. Control animals either on normal or choline-deficient, high-fat diets were injected with saline. Serotonin injections were started when the animals were 67 days old and were continued for a maximum of 353 days. The dosage was gradually increased from 2 mg. to a maximum of 8 mg. twice daily during the first 63 days and continued at this level for the remaining 290 days. Animals were killed at intervals and tissues were fixed and stained for histological examination.

The lesions found included necrosis of the tip of the tail and digits, tubular and some cortical necrosis of the kidneys, dermal fibrosis and cataracts. Animals with fatty livers appeared to be predisposed to kidney necrosis but serotonin did not seem to influence the severity of the dietary liver changes. *No lesions were observed in either myocardium or endocardium, including the valves.*

The kidney lesions that have been produced in growing rats on a choline-deficient diet are believed to be the result of compression of small vessels by fat-filled, tubular epithelial cells (W. S. Hartroft, *Brit. J. Exp. Path.* **29,** *483 (1948)*). Older animals do not ordinarily show these effects of a choline-deficient diet but in the present experiment in which serotonin injection was combined with choline deficiency, animals developed kidney lesions. Serotonin has a vasoconstrictive action and the combination of ischemic effects from two sources may account for the increased incidence of kidney lesions (similar to those seen in the

choline-deficient younger animals) in older animals receiving the combined treatment.

The absence of cardiac lesions may have been related to the test animal used, the route of administration, or the need for more than a normal load on the heart. It is also possible that serotonin or the form used in this experiment does not produce changes in the heart valves. The production of heart lesions could be due to some other metabolite in the pathway of tryptophan degradation. It would be interesting to determine the effect of serotonin injection in animals fed a tryptophan-deficient diet. The necrosis in the tip of the tail and the dermal fibrosis may be similar to those changes seen in niacin deficiency and may reflect the close relationship between tryptophan and niacin metabolism.

In summary, Rats given 5-hydroxytryptamine either with a normal or high-fat, choline-deficient diet were compared with control animals getting these same diets but with saline injections. The morphologic lesions which developed included dermal fibrosis, necrosis of the tip of the tail, necrosis of the tips of the digits, renal tubular necrosis and lens opacity.

TRANSPORT OF FATTY ACIDS

The way in which fat is absorbed from the intestine has been studied in detail in many laboratories (*Nutrition Reviews* **8,** *300 (1950)*; **9,** *211, 356 (1951)*; **10,** *54 (1952)*; **13,** *25 (1955)*; **15,** *71 (1957)*) and, although much remains to be learned about the mechanism of fat absorption, there is a good deal of information about the types of lipids which appear in the lymph and in the portal blood after the ingestion of fat. Much is also known about the composition of blood lipids in the post-absorptive state. There is, however, much less information about the method of transport of newly ingested fatty acids in the blood, but this subject has been receiving more attention in recent years (*Ibid.* **11,** *85, (1953)*).

Using as a base the observations of Borgström and asscciates (*Acta physiol. Scandinav.* **25,** *120 (1952)*; **29,** *264 (1953)*) that blood lipids in the post-absorptive state appear to originate in the liver, J. F. Mead and D. L. Fillerup (*J. Biol. Chem.* **227,** *1009 (1957)*) have administered isotopically labeled fatty acids to rats and have studied the distribution of the isotope in the blood at various times after ingestion. Carboxyl-labeled methyl stearate, methyl oleate and methyl linoleate were prepared and purified chromatographically. They were diluted with corn oil and administered orally to male Wistar rats. Blood samples were removed at one-half, one, two, four, and twenty-four hours after administration of the labeled fatty acids. The erythrocytes were separated and the total lipids of plasma and erythrocytes were extracted and fractionated using silicic acid columns. The lipid fractions were weighed and the C^{14}-content of each fraction was determined. The fractions determined included sterols, sterol esters, triglycerides, phospholipids and in some trials an unknown fraction. From two to four rats were used with each fatty acid at each time interval. The erythrocytes contained some labeled fatty acid as early as 0.5 hour after the oral administration of the fat. There appeared to be little relationship between plasma and erythrocytes. Also the distribution of the label in the erythrocyte fractions was highly variable, so that the major emphasis was placed on the results obtained with the plasma fractions.

The distribution of the radioactivity among the various fractions was much the same after both stearate and oleate administration. At 0.5 hour 80 to 90 per cent of the activity was in the triglyceride fraction. This decreased gradually (more rapidly with stearate than with oleate), until at 24 hours only 6 per cent of the activity from stearate was found in this fraction and 33 per cent

of the activity from oleate. The activity of the sterol fraction remained relatively constant and small in both cases, but the activity of the phospholipids and sterol ester fractions increased gradually as that of the triglyceride fraction fell.

Within a short time after linoleate administration the major portion of the radioactivity was found in the phospholipid fraction, and this remained fairly constant throughout the 24 hour experimental period. Again the activity of the sterol ester fraction increased with time at the expense of that of the triglyceride fraction.

One anomalous feature of the results was that the cholesterol fraction contained more radioactivity than would have been expected if the activity had arisen from its biosynthesis from breakdown products of the labeled fatty acids. Further purification of the sterol fraction revealed that two highly labeled fractions could be separated from cholesterol and that the cholesterol itself was only slightly labeled. These fractions were identified as mono- and diglycerides.

In a subsequent experiment rats were given a fairly large amount of radioactive methyl oleate and after two hours they were exsanguinated and the entire plasma lipid was collected. Unlabeled mono- and dipalmitin were added to the plasma to facilitate chromatographic recovery of the labeled mono- and diglyceride fractions, and separation of the various fractions was carried out. Both the mono- and the diglyceride fractions were active and contained an amount of C^{14} approaching that in the triglyceride fraction. The procedure was shown not to break down triglycerides. Therefore, these fractions could not have been artifacts produced by the experimental methods.

The observations provide a further demonstration of the rapidity of fat digestion and absorption. The authors discuss their results in relation to what is currently known about lipid absorption and metabolism, pointing out that the rapid appearance of oleate and stearate as triglyceride would be expected since they are known to pass as triglycerides from the lymph to the blood (B. Borgström, *Acta physiol. Scandinav.* **25,** *140, 315 (1952)*; Borgström *et al. Biochem. J.* **58,** *600 (1954)*). The gradual drop in the radioactivity of the triglycerides with the concurrent increase in the radioactivity of the phospholipids suggests that this conversion occurs largely in the liver. The similarity between the results obtained with stearate and those with oleate was striking, suggesting that oleate is handled by the body in the same way as are saturated fatty acids. Linoleate, the only essential fatty acid examined, was obviously handled differently. It was more rapidly incorporated into phospholipid and went to a greater extent into cholesterol esters, suggesting an intimate role for essential fatty acids in cholesterol transport. The authors suggest also that the rapid incorporation of linoleate into phospholipids may be indicative of a role of essential fatty acids in stabilizing lipoproteins and, thereby, facilitating fat transport to sites of oxidation and deposition.

Perhaps the most interesting observation made by Mead and Fillerup is that, after the ingestion of fat, relatively large amounts of fatty acids can be detected in the mono- and diglyceride fractions of the blood. They suggest three possible sources of these fractions: 1) Absorption of partially hydrolyzed glycerides; 2.) partial hydrolysis of triglycerides in the blood; 3.) partial hydrolysis of triglycerides in the tissues with the return of these to the blood.

Although mono- and diglycerides are known to be formed during fat digestion (*Nutrition Reviews* **14,** *88, 308 (1956)*) there is little evidence that they appear as such in the lymph (Reiser and Williams *J. Biol. Chem.* **202,** *815 (1953)*). Also there is, at present, no evidence to support either of the other alternatives. It is an important observation, nevertheless, that the mono- and diglyceride fractions may make up a considerable proportion of the lipids in the blood after a meal containing fat.

DIETARY FAT, OBESITY AND LONGEVITY

It has been reported that a high-fat diet can lead to obesity in rats even in the absence of any other factors (*Nutrition Reviews* **14,** *189* (*1956*)). Moreover, very obese rats have been found to have somewhat shorter life-spans than normal animals. Since different fats, when fed at a high level, have a considerably different effect on growth promotion (*Nutrition Reviews* **14,** *305* (*1956*)) and since a greater sustained growth rate appears to lead to an earlier death (*Nutrition Reviews* **14,** *349* (*1956*)), it is important to study the effect of different common dietary fats in promoting obesity.

Such a study has been carried out by J. J. Barborak, W. A. Krehl, G. R. Cowgill and A. D. Whedon (*J. Nutrition* **64,** *241* (*1958*)). Over 600 young rats were divided into seven series depending on initial weight (35 to 95 g.) and subdivided into groups of 12 to 40. Different groups were placed on either a high carbohydrate diet containing 65.6 per cent dextrin, 5 per cent corn oil and 5 per cent animal and vegetable shortening or a high fat diet in which these components were replaced by 61.6 per cent of a particular fat. The fats used in this study were three liquid oils (corn oil, coconut oil and cottonseed oil) and four higher melting fats (lard, butter, a hydrogenated vegetable shortening and margarine).

Within three weeks, it became apparent that animals on the solid fat diets were gaining weight more rapidly than were those on the vegetable oil diets, with the control animals falling in between these groups. The differences persisted throughout the experiment (up to two years) with the greatest gains recorded in the lard and crisco groups followed by the butter and margarine groups of the solid fat diets and corn oil for the vegetable oils. Eighty per cent of the animals on the lard diet ultimately weighed 1000 g., the heaviest reaching 1450 g. All animals on the high-fat diets were more or less obese.

Although the data are not given, the authors state that longevity appeared to be related to the weight at which animals were placed on the diets, the animals which were started at younger ages having a shorter life span. For series seven it is stated that the shortest average life span was found in the lard group and the longest in the control group. In the experiments of H. J. Thomasson (*J. Nutrition* **57,** *17* (*1955*); **59,** *343* (*1956*)), longevity appeared to be inversely related to growth rate, the highest rate being found on a high butter fat diet and the lowest on a rapeseed oil diet. Growth rate in turn was found to be directly related to rate of intestinal absorption of the fat. In the present experiments, a somewhat different interpretation may have to be made. Although the control animals on the low fat diet gained more rapidly during the first three weeks of the experiment, their relative rate was lower for the remainder of the experiment, and they were the least obese of the older groups of animals. Thus it appears that a lowered longevity may be related more to total weight gain or later growth rate rather than to initial growth rate. However this paper is largely devoted to the differences in weight gain produced by the different dietary fats. No analysis of the data on length of life is presented. As in the experiments of Thomasson (*Loc. cit.*) no unusual pathological changes were noted in any of the groups, and certainly nothing appeared to explain a decreased longevity.

These experiments emphasize again that, at least in rats, increased growth rate leading to obesity can be brought about by a high fat diet with no other manipulation and that these conditions may lead to a decreased life span.

NOTES

Breast Feeding in the United States

A companion study to the survey concerning current practices of artificial feeding of newborn infants by H. F. Meyer (*Nutrition Reviews* **16,** *233 (1958)*) is a compilation of similar data concerning the incidence of breast feeding in the same group of infants (*Pediatrics* **22,** *116 (1958)*). These data were obtained by questionaire from 1,904 hospitals and reflect the percentage of infants in their newborn nurseries that were breast fed at the time of discharge. These data are compared with those accumulated by K. Bain in 1946 (*Pediatrics* **2,** *313 (1948)*). The data are expressed as the per cent of infants leaving the hospital on any particular feeding and are broken down by regions. Both studies apparently covered essentially the same hospitals.

The results indicate that there are more infants leaving the hospital on artificial feeding now than there were ten years ago. This was true in all of the major geographical regions of the United States. The greatest number of artificially fed infants (79 per cent) was in the northeast, as was the case ten years previously with 61 per cent. The southwest had the least number artificially fed (50 per cent), as was true a decade ago (18 per cent). The state of Mississippi had the greatest increase in artificial feeding from 14 per cent ten years ago to 74 per cent at present. Tennessee, Arizona, and New Mexico had the lowest incidence of artificial feeding, and Massachusetts, Connecticut and Maine the highest in the present survey. The overall increase in artificial feeding was from 35 per cent in Bain's survey to 63 per cent in the present survey.

In the more recent survey 84 per cent of the infants left the hospital at the age of five days or less. Meyer suggests that there may be a relationship between early discharge of the mother and infant and the failure to breast feed; however, there are no data concerning this relationship.

In conclusion Meyer is cautious in interpreting these data as an inclination in the decrease of breast feeding in this country and points out that this cannot be considered to be a trend unless it is confirmed by comparable surveys ten or twenty years hence. Despite considerable current medical and lay interest in promoting the incidence of breast feeding in this country, the available statistics suggest that little progress has been made.

Fatty Acids and Protein Digestion

It has been shown that while fibrous proteins are readily digested by trypsin in both the native and denatured state, some of the globular proteins, such as egg albumin, require denaturation before tryptic hydrolysis can proceed (F. Haurowitz, M. Funca, R. Schwerin and V. Gokso, *J. Biol. Chem.* **157,** *621 (1945)*). Thus it is anticipated and found that while meat is readily digested both raw and cooked, egg white is not readily attacked by trypsin unless it is denatured by prior boiling.

Having reconfirmed these results and found that maximum digestion of egg white occurred after treatment at 80° for 10 minutes, Kondo (*J. Biochem.* **45,** *151 (1958)*) considered the possibility that other factors in the food might normally aid in the digestion of egg white. Since fatty acids are normally present in the food, the effect of these on the tryptic digestion of ovalbumin was first ascertained. For these experiments, the ovalbumin solutions were incubated at 37° for various times with the trypsin solution, phosphate buffer and several concentrations of the fatty acids (as sodium salts). With no fatty acid present, only 7.5 per cent of the raw albumin had been digested in 40 minutes as compared with 59.2 per cent of the albumin denatured at 80° and 36.7 per

cent denatured at 98°. N-caproic acid (C_6) did not increase the rate of digestion appreciably in concentrations up to 0.4 M. N-caprylic acid (C_8) produced 50.2 per cent hydrolysis in 40 minutes at a concentration of 0.15 *M*; n-capric acid (C_{10}) gave a 55 per cent hydrolysis at 0.05 *M*; and lauric acid (C_{12}) gave a 53.5 per cent hydrolysis at 0.015 *M*. Fatty acids with longer chains were less effective than lauric, and all fatty acids inhibited hydrolysis at high concentrations, possibly by denaturing the trypsin.

In further experiments, Kondo determined the alterations in the specific rotation of the albumin obtained by addition of the fatty acids. In all cases an increase in laevo rotatory power of the solutions was obtained, the maximum again being with lauric acid. This change is in the same direction as that obtained with heat denaturation.

At the present time it is difficult to assess these results from the point of view of practical nutrition. It is probable that in normal individuals some peptic digestion may render the egg white more susceptible to tryptic hydrolysis provided it remains in the stomach for a sufficient period. Although no fatty acids higher than myristic (C_{14}) were used in these experiments, its activity was still great enough to lead to the conclusion that palmitic and even stearic acids might show the effect provided they were present in sufficiently high concentrations.

Lipotropic Factors

A report of a symposium on the mode of action of lipotropic factors in nutrition occupies 134 pages of the May–June number of *The American Journal of Clinical Nutrition* (**6,** *197 (1958)*). This symposium was held at the University of Pittsburgh on October 22–23, 1957 under the chairmanship of Dr. R. E. Olson. The report consists of 12 papers, including the subsequent discussions, and represents the contributions of some 30 or more scientists whose work has contributed to our knowledge of the nutritional and metabolic aspects of liver fat deposition. The papers are, in general, well documented and should serve not only as a comprehensive and up-to-date survey of the subject for classroom lectures but also as a compact source of reference material for those actively engaged in research. Among the topics covered are the biosynthesis of choline, betaine and phospholipids; the effect of choline on hepatic fat oxidation and on the turnover of phospholipids; the effect of nutritional factors, including antagonists, on renal lesions and liver fat deposition; and observations on the effects of lipotropic factors on vascular disease, serum lipids, serum cholesterol, and fatty livers in man and in experimental animals. The preparation of formal papers adds considerably to the demands on the participants of symposia such as this, but publication of these papers enables many who are unable to attend the meetings to benefit from their colleagues' effort.

Recent Nutrition Books

Resource Training for Business, Industry, Government. Published for The Natural Resources Study Committee by the Conservation Foundation, 30 East 40th Street, New York 16, N. Y. 1958. Pp. 158.

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